IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Ian Garner, Michael A. Dalrymple, Donna E. Prunkard, Donald C. Foster
Serial No. : 08/206,176
Filed : March 3, 1994
For : PRODUCTION OF FIBRINOGEN IN TRANSGENIC ANIMALS

Examiner : Stanton, B.
Art Unit : 1804
Docket No.: 93-15
Date : May 3, 1995

Assistant Commissioner of Patents and Trademarks
Washington, D.C. 20231

Declaration of Gerald W. Lasser Under 37 C.F.R. § 1.132

Sir:

I, Gerald W. Lasser, hereby declare as follows:

1. I am an Associate Scientist at ZymoGenetics, Inc., an assignee of the above-identified application.

2. I have read and understand the specification and claims of the above-identified application.

3. The Experiments described in this Declaration were performed by me or by those under my direct supervision.

4. I have read the Office Action dated February 6, 1995 in the subject application, including the objection and rejection under 35 U.S.C. § 112, first paragraph, and provide this Declaration for the purpose of assisting the

Examiner in evaluating the teachings of the specification with regards to the production of biocompetent fibrinogen.

5. Experiments were carried out to characterize fibrinogen produced in the milk of transgenic mice. The mice had been generated by microinjection of fibrinogen subunit DNA constructs as disclosed in the subject patent application. Fifty μl of milk was diluted three-fold (150 μl final volume) with TBS (25 mM Tris pH 7.4, 100 mM NaCl) and centrifuged to remove casein. The supernatant was recovered and concentrated to a volume of 100 μl using a 10,000 molecular weight cutoff membrane. Two 20-μl samples of this concentrate were taken for subsequent analysis.

6. To characterize the fibrinogen, thrombin (5 U/ml) and $CaCl_2$ (10 mM) were added to one sample, and to the other sample were added thrombin, $CaCl_2$ and factor XIII (66 μg/ml). Both samples were incubated at 37°C for 30 minutes, then vortexed to condense any clotted fibrin. A small sticky ball was seen on the side of each tube after vortexing.

7. The condensed fibrin (sticky ball) was removed from each tube and dissolved in 10 μl of 10 M urea, 1% SDS, 1% 2-mercaptoethanol at 37°C for one hour. The starting concentrate and the 2 dissolved fibrin preparations were analyzed by gel electrophoresis and compared to a γ'-enriched fibrinogen standard by western blot analysis using a rabbit polyclonal antibody. This analysis demonstrated the removal by thrombin of fibrinopeptides A and B from the transgenic fibrinogen, as evidenced by a shift in molecular weight of the α and β bands. Both transgenic fibrinogen preparations were also found to contain γγ dimer, indicating the presence of transglutaminase-catalyzed cross-links (and indicating that a tranglutaminase was present in the mouse milk).

8. On the basis of these experiments, I readily conclude that the transgenic mouse milk contained fibrinogen made up of α, β and γ chains, and that the fibrinogen was in a biologically functional form that could be converted by thrombin to fibrin polymers that were subsequently cross-linked by factor XIII.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

Gerald W. Lasser

Gerald W. Lasser

Date: 5-3-95